Jantakoson *et al. International Aquatic Research* 2012, **4**:19
http://www.intaquares.com/content/4/1/19

ORIGINAL RESEARCH Open Access

Effect of high pressure and heat treatments on black tiger shrimp (*Penaeus monodon* Fabricius) muscle protein

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Abstract

Application of high pressure, a minimal processing, has gained interest particularly in extending the shelf-life or modifying the texture of seafood, especially in shrimp. However, pressurization may render the products with different texture as compared with their fresh and heated counterparts. Therefore, the impact of high-pressure treatment (200, 400, 600, and 800 MPa for 20 min at 28°C) on black tiger shrimp muscle proteins in comparison with heat treatment (100°C for 2 min) was investigated. Differential scanning calorimetry thermogram indicated that high pressure up to 200 MPa for 20 min induced the denaturation of myosin and actin with subsequent formation of a network stabilized by hydrogen bond. An electrophoretic study revealed that the sample pressurized at 800 MPa or heated at 100°C was also stabilized by disulfide bond. L^*, a^*, and b^* values, compression force, and shear force increased with increasing pressure ($p < 0.05$). The heat-treated sample had higher L^*, a^*, b^*, and shear force (toughening) than the pressurized and fresh samples ($p < 0.05$). Pressure at different levels had no effect on weight loss ($p \geq 0.05$). However, a weight loss of 27.89% was observed in the heat-treated sample. Proteolytic activity of crude extract from the pressurized sample at 200 to 600 MPa did not differ from that of the fresh sample ($p < 0.05$). Nevertheless, the activity in the heated sample and that of the pressurized sample at 800 MPa decreased, indicating the inactivation of endogenous proteases in the muscle.

Keywords: High pressure, Heat, Shrimp, Muscle protein, Protease activity, Texture, DSC, SDS-PAGE

Background

Thailand is the world's leading shrimp farming country and has become the top supplier, accounting for 90% of the global aquaculture shrimp production (Nirmal and Benjakul 2012). Black tiger shrimp (*Penaeus monodon* Fabricius) is one of the economically important species and is commonly cultured (Cheejareon et al. 2011).This high-value shrimp is very perishable and susceptible to deterioration during handling and storage. With increasing consumer demand of safe foods, which are minimally processed, additive free, and shelf stable, nonthermal food processing techniques such as ozone treatment, pulsed electric fields, ultraviolet radiation, oscillatory magnetic field, and high pressure are gaining interest. Among these various techniques, high pressure has shown a potential for producing food of natural characteristics when applied alone or in combination with other

processing methods (Paarup et al. 2002; Lakshmanan et al. 2003; Murchie et al. 2005; Pérez-Won et al. 2005). The main advantage of high-pressure technology, particularly in seafood products, is that this treatment generally extends the shelf-life by controlling or inactivating enzymes related to food deterioration, destroying both spoilage microorganism and food-borne pathogens, modifying texture, stabilizing color, and retarding lipid oxidation (Lakshmanan et al. 2003; Briones et al. 2010; Erkan et al. 2010).

High pressure causes protein denaturation and leads to inactivation of enzymes. Fish enzymes are generally more susceptible to hydrostatic pressure inactivation than their mammalian counterparts (Ashie and Simpson 1996). The degree of inactivation depends on the pressure levels, pressurization time, and type of enzyme (Hurtado et al. 2001a). (Hurtado et al. 2001a, 2001b, 2001c), revealed that autolytic activity and microbial loads of octopus (*Octopus vulgaris*) muscle drastically decreased when pressure over 200 MPa (7°C or 40°C, 15 or 30 min) was applied. However, (Hernández-Andrés et al. 2005) showed that pressure treatment (300 MPa, 7°C, 20 min) increased the proteolytic activity of squid (*Todaropsis eblanae*) mantle. (Tironi et al. 2010) observed the denaturation of sea bass myosin and sarcoplasmic protein when either a pressure-shift freezing or thawing process at 200 MPa was performed. Moreover, (Angsupanich and Ledward 1998) showed that the pressure-treated cod muscle was harder, chewier, and gummier than the cooked product. Different profiles in cod muscle texture were primarily due to the different responses of myofibrillar protein to heat and pressure, as well as the different survival rates of proteolytic enzymes in both heated and pressure-treated samples A similar effect of high pressure was also found in sea bream (*Sparus aurata* L.) (Campus et al. 2010), rainbow trout (*Oncorhynchus mykiss*), and mahi mahi (*Coryphaena hippurus*) (Yagiz et al. 2007). Other reports revealed an increase in cohesiveness and chewiness of pressurized abalone (550 MPa 8 min, or 500 MPa 3 to 5 min) (Briones-Labarca et al. 2012) as well as in the shear strength of pressurized prawn (*Penaeus japonicas*) (López-Caballero et al. 2000). Nevertheless, (Pérez-Won et al. 2005) showed that the hardness of bay scallop (*Aeuipecten irradians*) adductor decreased when pressurized at 200 or 400 MPa for 10 min. In addition, high-pressure treatment resulted in a glossier, smoother, and whiter adductor muscle in bay scallop (Pérez-Won et al. 2005) as well as that in prawn (López-Caballero et al. 2000), cod (Angsupanich and Ledward 1998), rainbow trout, and mahi mahi (Yagiz et al. 2007) compared with their unpressurized counterparts.

Although there are many reports on the application of high-pressure technology to seafood products, the use of high pressure in shrimp, particularly in fresh black tiger shrimp (*P. monodon* F.), is still limited. Therefore, the purpose of this paper was to study the effect of high pressure compared with heat treatment on protein denaturation and textural property of black tiger shrimp.

Methods

Chemicals

Trichloroacetic acid (TCA) was purchased from Merck (Darmstadt, Germany). L-Tyrosine, sodium dodecyl sulfate (SDS), β-mercaptoethanol (βME), glycerol, Coomassie Brilliant Blue R-250, and high molecular weight markers were obtained from Sigma-Aldrich (St Louis, MO, USA). *N,N,N′,N′*-Tetramethyl ethylene diamine, bis-acrylamide, and urea were procured from Fluka (Buchs, Switzerland). All chemicals used were of analytical grade.

Collection and preparation of shrimp

Black tiger shrimp (*P. monodon* F.) with a size grade of 60 shrimps/kg were obtained from the dock in Songkhla province, Thailand (24 to 48 h after capture). The shrimps were kept in ice with a shrimp-to-ice ratio of 1:2 (*w*/*w*) and transported to the Department of Food Technology, Prince of Songkla University within 1 h. Eight shrimps (110 to 120 g) were washed, headed, peeled, placed in 4 × 16-cm nylon laminated with low-density polyethylene bags, and heat-sealed.

The samples in seal bags were subjected to different treatments. Some portions were subjected to a high-pressure rig (S-FL-850-9-W model, Stansted Fluid Power Ltd, Essex, UK) using a mixture of ethanol and castor oil (1:4) as the pressure medium increased at 200, 400, 600, and 800 MPa for 20 min at 28°C. The rest of the samples were heated in boiling water for 2 min and immediately cooled in ice. The control comprised an unpressurized sample (0.1 MPa). All samples were subjected to analyses as follows.

Protease activity

Shrimp samples (100 g) were homogenized with 300 ml of 20 mM phosphate buffer (pH 7) using an IKA Laboratechnik homogenizer (NISSEI-AM-8, Salangor, Malaysia) for 2 min at a speed of 11,000 rpm. Homogenate was centrifuged using an Rc-plus Sorvall centrifuge (Sorvall, Newtown, CT, USA) at 17,500 × *g* for 20 min at 4°C. The supernatant was referred to as 'crude extract' and was determined for protease activity using the Casein-TCA Lowry method (An et al. 1994). Crude extract (250 μl) was added to the preincubated reaction mixture containing 200 μl of 2% (*w*/*v*) casein, 200 μl of distilled water, and 625 μl of 0.2 M McIIvaine's buffer (0.2 M disodium hydrogen phosphate and 0.1 M sodium citrate, pH 7). The mixture was incubated at 60°C for 1 h. Optimized conditions for proteolytic activity of crude extract from black tiger shrimp muscle were pH 7 and 60°C using casein as substrate (data not shown). Enzymatic reaction was terminated by adding 200 μl of 50% (*w*/*v*) TCA. Unhydrolyzed protein substrate was allowed to precipitate for 15 min at 4°C and centrifuged at 6,500 × *g* for 10 min. The oligopeptide content in the supernatant was determined using the Lowry method (Lowry et al. 1951) with tyrosine as a standard. One unit of activity was defined as that releasing 1 mmol of tyrosine per minute (mmol/Tyr/min). A blank was run in the same manner, except that the enzyme was added after addition of 50% TCA (*w*/*v*).

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed on a PerkinElmer differential scanning calorimeter DSC 7 (Norwalk, CT, USA). Samples of 10 to 20 mg at the middle of the second segment of the abdomen were sealed into an aluminum pan hermetically. The sample pan and reference pan (empty pan) were heated from 10°C to 95°C at 10°C min^{-1}. The temperature at each endothermic peak was recorded. Denaturation enthalpies were estimated by measuring the corresponding areas under the DSC transition curve. Triplicate samples were analyzed.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis was performed according to the method of (Laemmli 1970). To 3 g of the sample,

27 ml of 5% (*w*/*v*) SDS solution was added. The mixture was then homogenized using an IKA Laboratechnik homogenates (NISSEI-AM-8, Salangor, Malaysia) for 2 min at a speed of 11,000 rpm and incubated at 85°C in a water bath for 1 h to dissolve total proteins. The sample was centrifuged at 5,500 × *g* for 15 min to remove undissolved debris. Solubilized samples were mixed at 1:1 (*v*/*v*) ratio with the nonreducing and reducing sample buffer (0.5 M Tris–HCl, pH 6.8 containing 4% SDS, 20% glycerol, without and with 10% βME, respectively) and boiled for 3 min. The samples (20 μg of protein) were loaded onto the polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel using a Mini Protein Π unit (BioRad Laboratories, Inc., Richmond, CA, USA). After separation, the proteins were stained with 0.02% (*w*/*v*) Coomassie Brilliant Blue R-250 in 50% (*v*/*v*) methanol and 7.5% (*v*/*v*) acetic acid and destained with 50% methanol (*v*/*v*) and 7.5% (*v*/*v*) acetic acid, followed by 5% methanol (*v*/*v*) and 7.5% (*v*/*v*) acetic acid.

Weight loss

To determine weight loss, eight shrimps were used for each measurement. After treatment, the shrimp samples were drained for 2 min at 4°C and patted dry with filter paper. Weight loss was calculated as the weight difference between sample before and after treatments using the following equation:

$$\text{Weight loss}\ (\%) = \left[(\text{Weight before treatment} - \text{Weight after treatment})/\text{Weight before treatment}\right] \times 100.$$

Triplicate determinations were performed.

Shear force and compression force

Shear force and compression force of the samples were measured at the second segment of the shrimp abdomen using a texture analyzer (TA-XT2i model, Stable Micro System, Surrey, UK). Shrimp samples were patted dry on the surface with filter paper and kept at 4°C for 30 min before they were analyzed. Shear force, perpendicular to the axis of muscle fibers, was carried out by cutting at the second segment of the shrimp abdomen with Warner-Bratzler blade using a cross-head speed at 2 mm·sec^{-1} and a 25-kg load cell. The peak of the shear force profile was regarded as the shear force value. Compression force was analyzed by compressing the samples to 5 mm for 9 min using the cylindrical-shaped probe with 6 mm in diameter. Six determinations were performed.

Color

Shrimps were patted dried on the surface with filter paper. Surface color at the middle part of the shrimp was measured by Hunter Lab (Colorflex, Reston, VA, USA). CIE L^*, a^*, and b^* values were recorded. Six replicate measurements were carried out.

Statistical analysis

All experiments were run in duplicate using two different lots of shrimp. Completely randomized design was used. Data were subjected to analysis of variance, and mean

comparisons were carried out by Duncan's multiple range test at a significant level of $p < 0.05$. Analysis was performed using the SPSS statistic program (version 10.0 for Windows) (SPSS Inc, Chicago, IL, USA).

Results and discussion

Effect of high pressure and heat treatment on protease activity of black tiger shrimp meat

The pressure ranging from 200 to 800 MPa had no significant effect on the activities of the protease extracted from black tiger shrimp ($p > 0.05$). The result suggested that these ranges of pressure were not able to inactivate the endogenous protease in the shrimp muscle. However, protease extracted from the heated sample appeared to decrease significantly ($p < 0.05$) as shown in Figure 1. (Manheem et al. 2012) reported that crude protease from the hepatopancreas of Pacific white shrimp (*Litopenaeus vannamei*) sharply decreased at temperatures above 70°C most likely due to thermal denaturation. Proteolytic activity from seafood including, cod (Angsupanich and Ledward 1998) and octopus (*O. vulgaris*) (Hurtado et al. 2001a) dramatically decreased when pressure was over 200 MPa. In addition, (Ashie and Simpson 1996) reported that the relative activity of cathepsin C, chymotrypsin, and trypsin from bluefish and sheephead decreased with increasing pressure (100 to 300 MPa for 30 min at ambient temperature). High pressure may affect enzymatic reactions directly by altering the kinetic constants or by changing the conformation of enzymes and/or substrates (Dufour et al. 1996). Pressurization conditions such as pressure level, time, temperature, and pH also play a role in either activation or inactivation of enzymes depending on the conditions and habitat temperature (Defaye and Ledward 1995; Low and Somero 1974).

Nevertheless, (Hernández-Andrés et al. 2005) demonstrated that pressure treatment (300 MPa at 7°C for 20 min) increased the proteolytic activity in squid (*Todaropsis eblanae*) mantle and resulted in an increased protein hydrolysis. (Jung et al. 2000) also showed that cathepsin D and acid phosphatase activities in pressurized meat (520 MPa at 10°C for 260 s) increased due to the breakdown of the lysosomal membrane and/or due to enzyme activation. Therefore, the pressurized sample from our results could undergo autolysis to a higher extent than the heated sample.

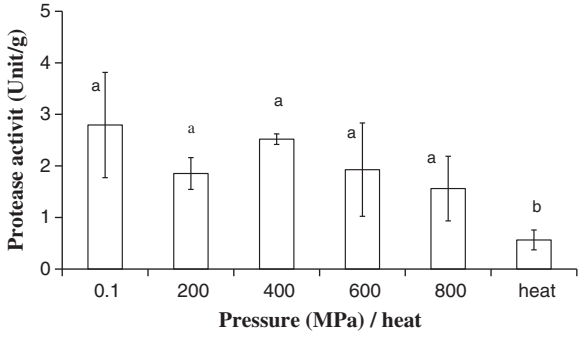

Figure 1 Influence of high pressure and heat treatment on protease activity of black tiger shrimp muscle. High pressure (200 to 800 MPa at 28°C for 20 min) and heat treatment (100°C for 2 min). The different letters on the bars indicate significant differences ($p < 0.05$). Bars represent the standard deviation ($n = 2$). For each run, triplicate determinations were conducted.

Effect of high pressure and heat treatment on thermal transition of black tiger shrimp muscle

Thermogram, T_{max}, and enthalpy (ΔH) of black tiger shrimp muscle and those pressurized at 200, 400, 600 and 800 MPa are presented in Figure 2 and in Table 1. For fresh shrimps, there were three endothermic peaks with T_{max} values of 48.6°C, 51.9°C, and 70.17°C, which were presumed to arise from myosin, sarcoplasmic protein with connective tissue, and actin, respectively. (Schubring 2005) revealed that the first peak at low temperature of the fish muscle (ocean perch, saithe, and herring) was connected with myosin and other related proteins, while the last at high temperature was connected with actin. The peaks between the above two were plausibly attributed to sarcoplasmic protein and/or collagen. It is similar to the work of (Srinivasan et al. 1997) who assigned the first peak at 50.5°C of intact fresh water prawn (*Macrobrachium rosenbergii*) to myosin transition. The second peak at 54.9°C was corresponded to the sarcoplasmic protein and connective tissue, and the last peak at 67.7°C was related to actin denaturation.

T_{max} (°C), enthalpy (joules/gram wet sample), and muscles (black tiger shrimp muscle). Peak $\underline{N}$ is assumed to be associated with a structure that is formed following myosin denaturation. Peak 1 corresponds to the first peak of myosin denaturation. Peak 2 corresponds to myosin, sarcoplasmic protein, and connective tissue denaturation. Peak 3 corresponds to actin denaturation

When the sample was treated at 200 MPa, both myosin and actin peaks disappeared. Heating at 100°C for 2 min caused the loss of all protein peaks (data not shown). It has been reported that myosin was denatured with increasing pressure, while actin became more resistant (Angsupanich and Ledward 1998; Angsupanich et al. 1999; Schubring 2005; Tironi et al. 2010). Our results showed that myosin and actin from black tiger shrimp muscle underwent thermal denaturation at 200 MPa. Thus, shrimp muscle was more sensitive to pressure than those from sea bass and cod muscles, which were denatured at higher than 200 MPa (Angsupanich and Ledward 1998; Tironi et al. 2010). This behavior appears to be species specific (Schubring 2005). In addition, it was found that at 200 MPa or higher, a new transition was detectable around 44°C to 45°C. (Angsupanich and Ledward 1998) suggested that the denaturation of myosin alone gave rise to the new transition which was not modified by further denaturation of sarcoplasmic protein, connective tissue, and actin. A new transition was dominantly stabilized by hydrogen bonds. Since high

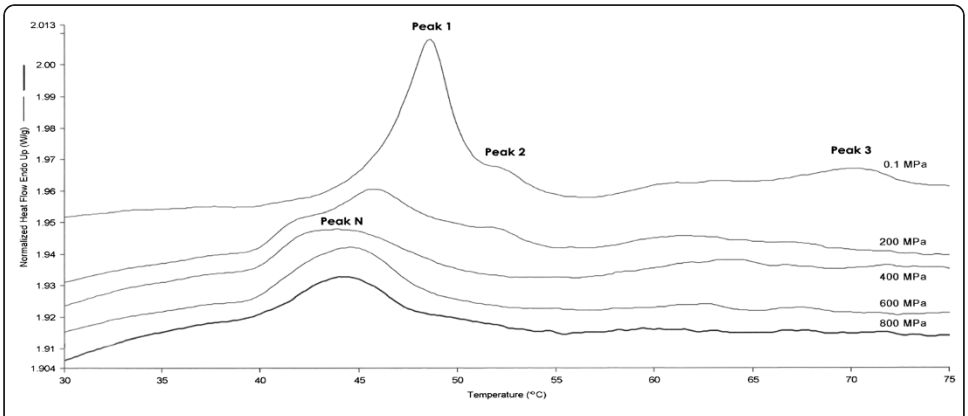

Figure 2 DSC thermograms of black tiger shrimp muscle subjected to different pressures for 20 min at 28°C. Heating rate was 10°C·min^{-1}.

Table 1 Effect of high pressure and heat treatments on T_{max} and enthalpy of protein denaturation in muscles

Treatment	Peak *N*		Peak 1		Peak 2		Peak 3	
	T_{max}	ΔH	T_{max}	ΔH	T_{max}	ΔH	T_{max}	ΔH
0.1 MPa	Not seen	0	48.67	1.314	51.96	0.01	70.17	0.128
200 MPa	45.83	0.920	Indistinct	Indistinct	51.79	0.02	Indistinct	Indistinct
400 MPa	44.00	0.165	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct
600 MPa	44.67	0.702	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct
800 MPa	44.33	0.607	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct
Heat	Not seen	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct

pressure was progressively exerted on protein, electrostatic and hydrophobic interactions would be readily broken. As pressure was further increased, hydrogen bonds were ultimately ruptured. However, as pressure was released, hydrogen bonds formed initially, and subsequently, hydrophobic and electrostatic bonds were superimposed on the structure.

Effect of high pressure and heat treatment on protein pattern of black tiger shrimp muscle

From protein patterns determined under nonreducing condition (Figure 3A), myosin heavy chain (MHC) band intensity decreased in the heated sample and that treated at

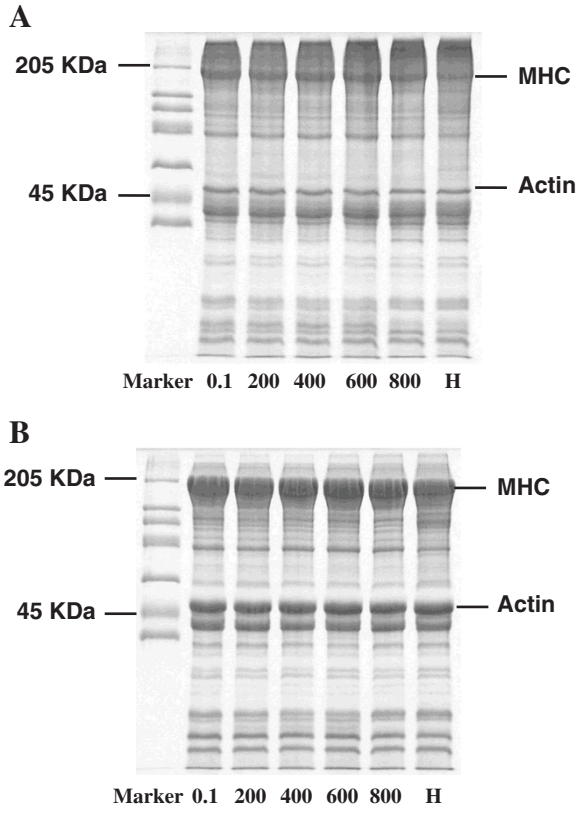

Figure 3 SDS-PAGE pattern of proteins determined under nonreducing condition (A) and reducing condition (B). Pattern is for black tiger shrimp muscle after undergoing different pressures (0.1, 200, 400, 600, and 800 MPa). *H*, heated sample; MHC, myosin heavy chain.

800 MPa, while actin and other bands remained unaltered. However, the protein patterns analyzed under reducing condition (Figure 3B) did not differ for all samples, especially for the heated sample and those pressurized at 800 MPa. Under reducing condition, βME disrupted the disulfide bond of those samples as indicated and retained more of the MHC band (Figure 3B). Thus, disulfide bonds were markedly formed during pressurization at high level or heating. When pressure is applied, the volume generally decreases, thereby favoring protein-protein interactions. The conformation changes at high pressure level and might lead to the increased formation of disulfide bonds via the oxidation of SH groups of myosin (Gilleland et al. 1997). For heat treatment, thermal condition results in the cleavage of the existing disulfide bond structure or the activation of buried sulfhydryl groups through unfolding protein. These activated sulfhydryl groups can form new intermolecular disulfide bonds which are essential for the formation of protein aggregates (Boye et al. 1997). The results are in agreement with those of (Angsupanich et al. 1999) who demonstrated that MHC from myosin and myofibrillar protein isolated from cod or turkey underwent disulfide bond formations when heated at 50°C and above or pressurized at 600 MPa and higher. It was noted that the band intensity of MHC and actin in the fresh sample and other samples was higher in the presence of βME. Thus, MHC and actin were stabilized by the disulfide bond in nature (Damodaran 1996).

Effect of high pressure and heat treatment on weight loss of black tiger shrimp

Figure 4 shows the effect of high pressure and heat treatments on weight loss of the shrimp muscle. Weight loss did not differ among the pressurized sample ($p > 0.05$). The highest weight loss was observed in the heated sample as compared with the pressurized sample ($p < 0.05$). Protein conformation changes induced by pressure might be lower than that induced by heat (Uresti et al. 2005). When protein is denatured, the water-binding capacity of protein decreases, as a result of decreased hydrogen bonding and decreased hydration of ionic groups (Damodaran 1996). During heating, protein underwent denaturation and aggregation, resulting in a corresponding decrease in the water-holding capacity due to the reduction in available space within the protein network for retention of water (Hamn 1986). This result was in agreement with that of the

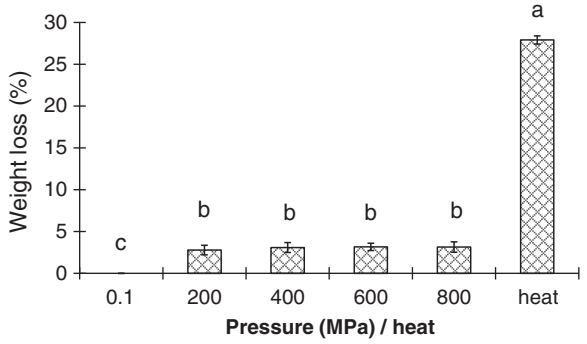

Figure 4 Effect of high pressure and heat treatment on shrimp muscle weight loss. High pressure (200 to 800 MPa at 28°C for 20 min) and heat treatment (100°C for 2 min). The different letters on the bars indicate significant differences ($p < 0.05$). Bars represent the standard deviation ($n = 2$). For each run, triplicate determinations were conducted.

work of (Angsupanich and Ledward 1998) who found that the moisture content of heated (70°C, for 10 min) cod muscle was lower than that of the pressurized (200 to 800 MPa for 20 min) sample. Therefore, pressurization could be applied for shrimp treatment and could reduce water loss from the muscle, which was done in the heated sample.

Effect of high pressure and heat treatment on texture of black tiger shrimp meat

The effect of heat and pressure at different levels on shrimp meat texture is depicted in Figure 5. Compression force and shear force increased with increasing pressure ($p < 0.05$). Among all samples, the heat-treated sample had higher shear force (toughening) than the pressurized and control samples. Similar results were reported by (Niamnuy et al. 2008) who demonstrated that the hardness of white shrimp (*Penaeus indicus*) meat increased as the boiling proceeded. Heat denaturation and aggregation of shrimp muscle proteins led to the shrinkage of both the filament lattice and collagen. The exposure of hydrophobic domains of myofibrillar protein allowed new intra- and interprotein interactions, thereby resulting in a dense protein structure. Pressure generally enhanced the hardness or shear strength of prawn (400 MPa at 7°C for 10 min) (López-Caballero 2000), black tiger shrimp (100 to 435 MPa at 25 ± 2°C for 5 min) (Kaur et al. 2012), octopus (200 to 300 MPa) (Hurtado et al. 2001a), abalone (550 MPa for 8 min or 500 MPa for 3 to 5 min) (Briones-Labarca et al. 2012), cod (Angsupanich and Ledward 1998). Hurtado et al. 2001a, Peréz-Won et al. 2005 and Briones-Labarca et al. 2012 suggested a relationship between the compacting of muscle fiber and increased muscle texture under pressure treatment. In addition, (Heremans 1997) stated that the increased in some texture parameters could be due to the reinforcement of hydrogen bonds of proteins caused by high pressure. Furthermore, (Lanier 1998) proposed that disulfide bonds were formed under pressure and that hydrogen bonds were formed upon the release of pressure. Moreover, (Angsupanich and Ledward 1998) found that cod muscle hardness increased with increasing pressure up to 600 MPa at 10°C for 20 min. This hardening of the muscle might be related with a new modified protein structure induced by high pressure, as evidenced by the formation of a new peak in DSC thermogram. (Angsupanich et al. 1999) also suggested that high pressure induced the formation of protein structure that contained hydrogen bond and were additionally stabilized by

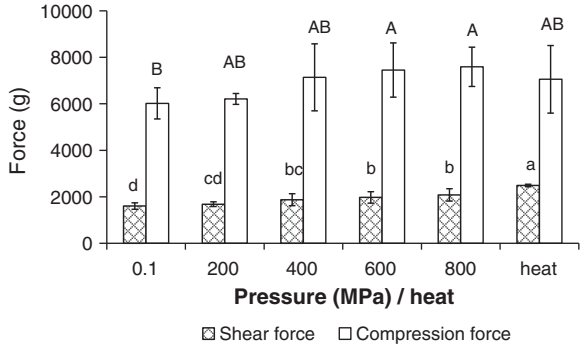

Figure 5 Influence of high pressure and heat treatment on shear and compression forces of shrimp muscle. High pressure (200 to 800 MPa at 28°C for 20 min) and heat treatment (100°C for 2 min). The different letters on bars or capital letters under the same parameter indicate significant differences ($p < 0.05$). Bars represent the standard deviation ($n = 2$). For each run, six determinations were conducted.

disulfide bonds, while heat treatment gave rise to a structure primarily stabilized by disulfide bonds and hydrophobic interactions. Furthermore, the different texture characteristics of both heated and pressure-treated samples might be determined by water retained in the sample. Since heat treatment caused losses of free water (Figure 4) at a higher extent when compared to that of the sample subjected to pressurization (200 to 600 MPa), this probably led to the toughening of shrimps subjected to heating.

Effect of high pressure and heat treatment on color of black tiger shrimp

The effect of high pressure and heat treatment on color (L^*, a^*, and b^*) of black tiger shrimp muscle is shown in Figure 6. Raw shrimps were gray and appeared slightly translucent. After pressurization at 200 MPa, shrimps turned bluish ($a^* = -3.20$) and slightly opaque. When the pressure increased, the sample became more opaque and whiter. At 800 MPa, the sample was slightly pink ($a^* = 3.26$), whereas the heat-treated sample turned orange red ($a^* = 24.63$). The cooked sample was more reddish in color than the control (unpressurized) and pressure-treated samples ($p < 0.05$). The results were coincidental with the black tiger shrimp meat gel, in which L^*, a^*, and b^* values of shrimp paste increased with increasing pressure (Cheejareon et al. 2011). (Paarup et al. 2002) also reported that the squid mantle skin color was changed from initial gray-brownish to reddish when pressure at 200 to 400 MPa was applied, due to rupture of chromatophores. The muscle of prawn (*P. japonicas*) (López-Caballero et al. 2000), black tiger shrimp (*P. monodon*; 100 to 435 MPa at 25 ± 2°C for 5 min) (Kaur et al. 2012), salmon (Lakshmanan et al. 2003), cod (*Gadus morhua*) (Angsupanich and Ledward 1998), red mullet (Erkan et al. 2010), rainbow trout (*O. mykiss*), and mahi mahi (*C. hippurus*) (Yagiz et al. 2007) became whiter when pressure was increased. The color of the muscular epithelium and exoskeleton of raw black tiger shrimp is expressed by blue carotenoprotein and deeply seated red carotenoids (astaxanthin esters) (Okada et al. 1995). When pressure at lower levels (200 to 400 MPa) was applied, the protein-carotenoid was partially dissociated and led to a slight release of free astaxanthin from the carotenoid complex. The denaturation of protein was more pronounced at higher pressure and color turned to be purple red (the overlapping between blue carotenoid and red astaxanthin). After the sample was subjected to heat treatment, the protein was totally denatured, and red color of astaxanthin and its derivatives became dominant.

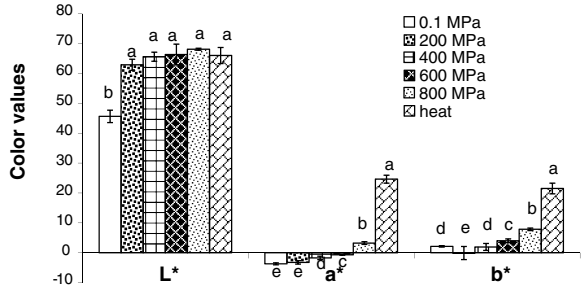

Figure 6 Effect of high pressure and heat treatment on color of black tiger shrimp muscle. High pressure (200 to 800 MPa at 28°C for 20 min), heat treatment (100°C for 2 min), and color (L^*, a^*, and b^*) The different letters on bars within the same color values indicate significant differences ($p < 0.05$). Bars represent the standard deviation ($n = 2$). For each run, six determinations were conducted.

Conclusions

Pressure (200 to 800 MPa at 28°C for 20 min) and heat treatments (100°C for 2 min) affected black tiger shrimp muscle proteins differently and led to different textural characteristics. The pressurized sample was stabilized with hydrogen bonds, while disulfide bond was the dominant bonding in samples that were pressurized at 800 MPa and heated. In addition, a decrease in autolytic activity and increase in weight loss found in the heated muscle might also contribute to those differences.

Abbreviations

DSC: Differential scanning calorimetry; *H*: Heat; MHC: Myosin heavy chain; MPa: Megapascal.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KK formulated the hypothesis and designed the study. TJ carried out the experiment and analyzed the data. KK and TJ prepared the manuscript. SB participated in the discussion and corrected the manuscript. All authors read and approved the final manuscript.

Acknowledgments

This work was supported by the Thailand Research Fund (MRG4780057) and the Graduate School, Prince of Songkla University.

Received: 13 September 2012 Accepted: 15 November 2012
Published: 5 December 2012

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doi:10.1186/2008-6970-4-19
Cite this article as: Jantakoson *et al.*: **Effect of high pressure and heat treatments on black tiger shrimp (*Penaeus monodon* Fabricius) muscle protein.** *International Aquatic Research* 2012 **4**:19.